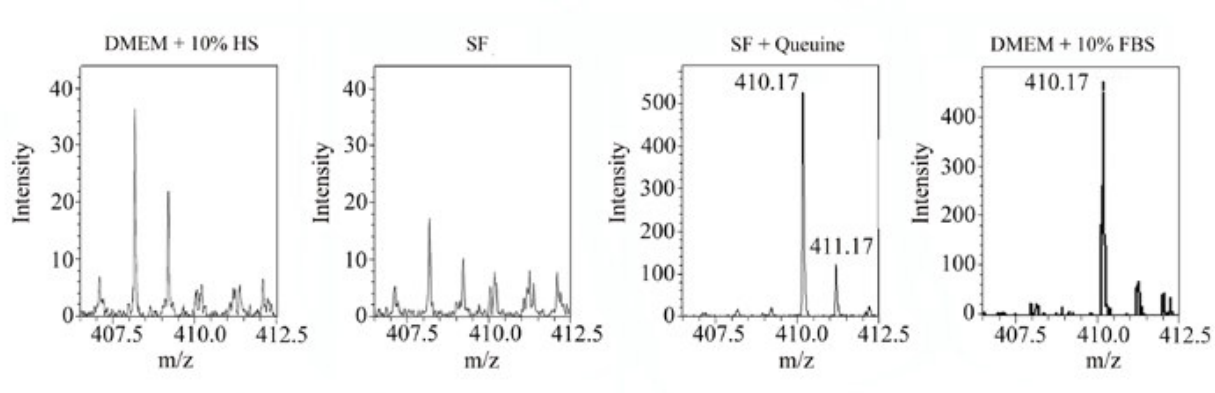
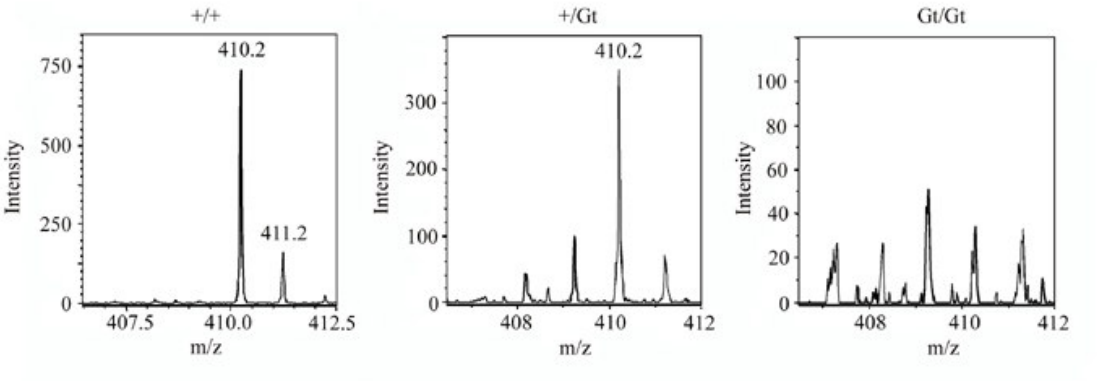
# Supplementary Figure 1



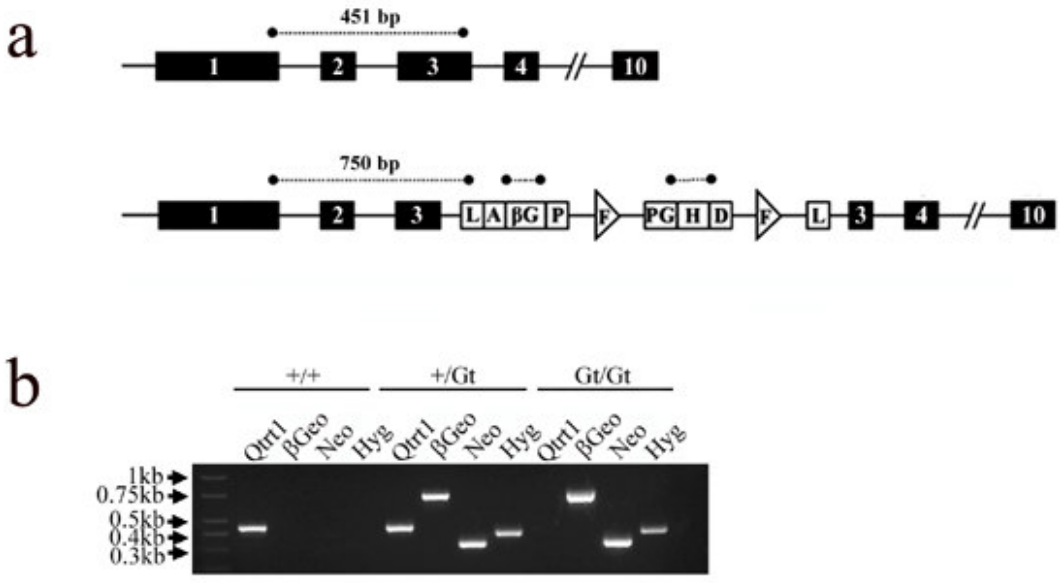

**Supplementary Figure 1: LC-MS analysis of Q-tRNA levels in HepG2 cells.** tRNA from HepG2 cells grown in various culture mediums was analysed by LC-MS to determine the presence or absence of the queuosine modification (410 m/z). DMEM supplemented with 10% horse serum (DMEM + 10% HS), Serum Free medium (SF), Serum Free medium plus queuine (SF + Queuine) and DMEM supplemented with 10% FBS (DMEM + 10% FBS).

# Supplementary Figure 2



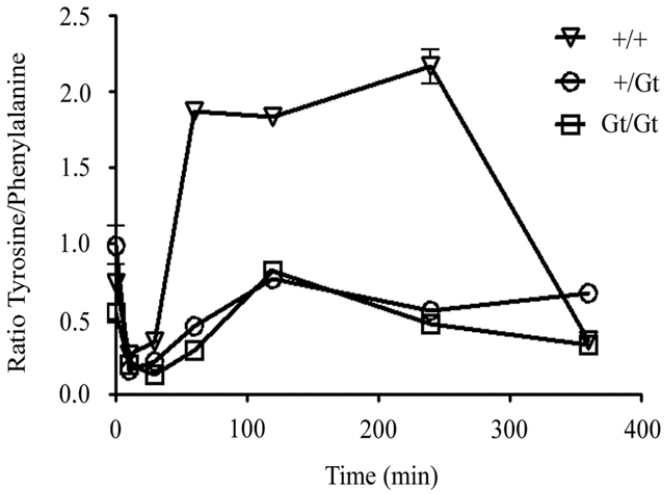

**Supplementary Figure 2: LC-MS analysis of Q-tRNA levels in liver of sixteen-week old mice.** Bulk tRNA was isolated from the liver of 16-week-old animals and enzymatically hydrolyzed before being analyzed by LC-MS. The queuosine peek is identifiable at 410 m/z.

# Supplementary Figure 3



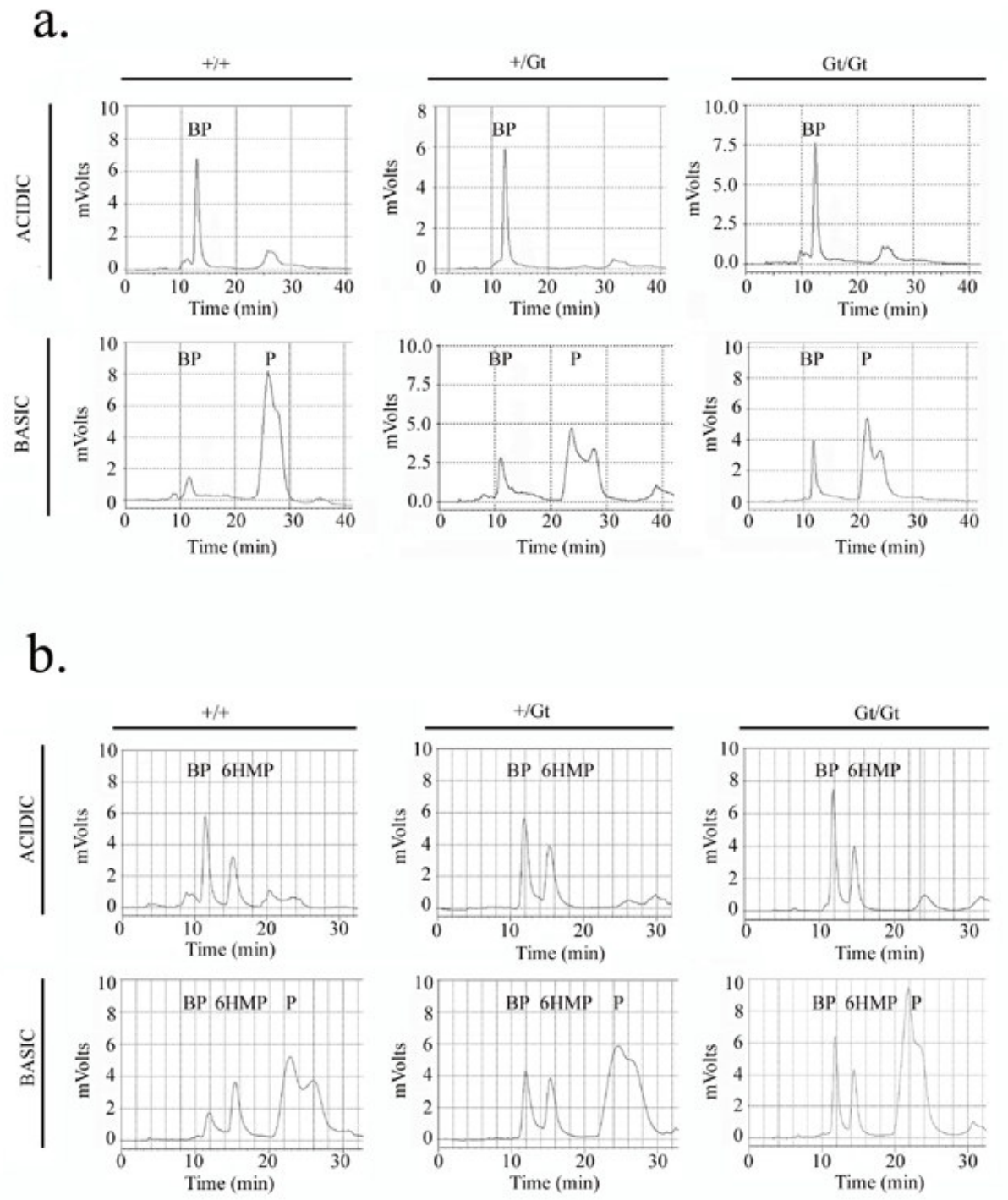

**Supplementary Figure 3: Genotyping strategy for *Qtrt1* gene-trap mice.** (A) Map of the wild type mouse *Qtrt1* exon structure (upper figure) represented by the solid black boxes and gene-trapped allele (lower figure) with a ROSAFARY gene-trap vector inserted within exon 3. The expected size of the PCR products for the wild type (451 bp) and gene-trapped allele (750 bp) are shown above each map. (B) Genomic DNA isolated from ear punches of 21-day-old mice was used in a PCR reaction to amplify specific regions within the *Qtrt1* wild-type allele and gene-trap allele. Amplicons from the QWF-QWR primer pair (451bp) and the QWF-QGR primer pair (750bp) were used routinely to differentiate between wild type (+/+), heterozygous (+/Gt) and homozygous (Gt/Gt) animals while PCR amplification using the NeoF-NeoR primer pair (323bp) and HYGF-HYGR primer pair (389bp) were used to confirm the presence of the neomycin and hygromycin resistance cassettes, respectively.

# Supplementary Figure 4




**Supplementary Figure 4: Phenylalanine to tyrosine conversion of fasted animals.** Food was withdrawn for twelve hours from eight individual wild-type (+/+), heterozygous (+/Gt) and homozygous (Gt/Gt) animals. Phenylalanine (1mg/g body weight) was administered i.p. and at the times indicated blood was withdrawn from the ventral caudal artery. Plasma phenylalanine and tyrosine were separated by HPLC on a reversed-phase C18 column and detected by their absorbance at 206 nm. Each point was measured in triplicate. The ratio of plasma tyrosine to phenylalanine is shown.

# Supplementary Figure 5



**Supplementary Figure 5: Representative HPLC chromatographs of pterins in the plasma and urine of *Qtrt1*$^{+/+}$, *Qtrt1*$^{+/Gt}$ and *Qtrt1*$^{Gt/Gt}$ mice.** (A) Blood was collected from the ventral caudal artery and plasma prepared to 3 mg/ml whereas (B) urine samples were diluted to a concentration of 30 µg/ml from wild-type (+/+), heterozygous (+/Gt) and homozygous (Gt/Gt) gene trap mice. Biopterin (BP) levels were measured by HPLC analysis after iodine oxidation under acidic or alkaline conditions by means of their native fluorescence ($\lambda_{ex}$=350nm and $\lambda_{em}$=450nm) and quantified by the area under the peaks using the EZStart 7.3SPI Chromatography integration software (Shimadzu). BH4 levels were derived by subtracting biopterin levels under a basic oxidative state (biopterin and 7,8 dihydrobiopterin) from the levels under an acidic oxidative state (Total biopterin; BH4, quinonoid dihydrobiopterin and 7,8 dihydrobiopterin). BP, biopterin; P, pterin; 6HMP, urinary 6-hydroxymethylpterin.

# Supplementary Table 1

| Mouse Group | Mice with indicated genotype (% of total) | | | Mice with indicated gender (% of total) |
|---|---|---|---|---|
| | +/+ | +/GT | GT/GT | |
| Female | 23 | 55 | 20 | 98 (43) |
| Male | 30 | 74 | 27 | 131 (57) |
| Total | 53 (23) | 129 (56) | 47(21) | 229 |

**Supplementary Table 1: Genotype and sex of progeny from *Qtrt1*$^{+/G}$ intercross.** A total of 36 litters (229 pups) were obtained from crossing Qtrt1$^{+/Gt}$ mice. PCR analysis, as described in Supplementary Fig. 3, was used to determine the distribution of wild type (+/+), heterozygous (+/Gt) and homozygous (Gt/Gt) progeny. The ratio of male to female for each genotype was also determined.

# Supplementary Table 2

| Mouse Group | Number of pups from cross of GT/GT male and GT/GT female mice (3 litters) | | | Mice with indicated gender (% of total) |
|---|---|---|---|---|
| Female | 3 | 2 | 6 | 11 (50) |
| Male | 4 | 3 | 4 | 11 (50) |
| Total | 7 | 5 | 10 | 22 |

**Supplementary Table 2: Analysis of the fecundity of Qtrt1$^{Gt/Gt}$ mice.** One male and three female Qtrt1$^{Gt/Gt}$ mice were mated producing 22 pups. The male:female distribution of the progeny was noted and their viability shown not to be affected for up to two-months of age.